

**Research paper****EFFECTS OF TRANEXAMIC ACID AND ITS DERIVATIVES ON THE CHEMICAL AND METABOLIC MODULATION OF GLUTATHIONE IN AQUEOUS SOLUTION**

A.H. KHAN\*, F. KHAN, Faculty of Pharmacy Gomal University, N.W.F.P, Pakistan,  
J. AKRAM, T. M. KHAN, M. A. HADI, School of Pharmaceutical Sciences, Universiti Sains Malaysia,  
11800 Minden, Pulau Pinang, Malaysia.

---

**ABSTRACT**

**PURPOSE** The aim of the present study was to examine the effects of Tranexamic acid and its derivatives, more specifically N-Acetyl-tranexamic acid on the metabolism and chemical modulation of Glutathione (GSH) in the aqueous solutions.

**METHOD** All the solutions were prepared in phosphate buffer solution pH 7.62 and absorbances were measured at 412 nm using U.V/ Visible spectroscopy.

**RESULTS** It was found that N-Acetyl-tranexamic acid had a marked effect on lowering of GSH and/or thiols in aqueous solutions as compared to the effect of Tranexamic acid and N-Phthaloyl-tranexamic acid.

**CONCLUSIONS** Finding provide evidence that N-Acetyl-tranexamic acid causes the depletion of GSH and/or thiols in aqueous solutions due to the oxidation.

**KEY WORDS** Tranexamic Acid, Glutathione, N-Phthaloyl-tranexamic acid, N-Acetyl-tranexamic acid

---

**INTRODUCTION**

The effects of Tranexamic acid and its derivatives i.e. N-Phthaloyl-tranexamic acid and N-Acetyl-tranexamic acid on the *metabolism and chemical modulation* of Glutathione (GSH) in the aqueous solutions is the novel work.

Glutathione (GSH) is a "NATURAL DRUG<sup>1</sup>". This naturally occurring compound is the most important and abundant sulfhydryl-containing compound in all tissues & hepatocytes of our body<sup>2-5</sup>. Its levels in our cells are predictive of how long we will live. Glutathione is called the "master antioxidant", It regulates the actions of lesser antioxidants such as vitamin C and E with the body. "We literally cannot survive without this antioxidant," Glutathione a naturally occurring tripeptide of three amino acids i.e. glycine, glutamic acid (glutamate), and cysteine. It protects every cell, tissue, & organ from toxic free radicals and diseases.

The concentrations of GSH in tissues varied depending on the need, number and kind of the metabolic processes requiring its presence. Example of GSH concentration in tissues is 15 mM in lens<sup>6</sup> and 5 to 10 mM in liver<sup>7</sup>. Liver is quantitatively the most important site of the glutathione synthesis. It plays a central role in providing glutathione for cellular protection against reactive intermediate and pollutant xenobiotics<sup>8,9</sup>. Blood is also one of the major site of GSH contents, Leukocytes contains 14 to 21 mM<sup>10</sup>, seven times more than erythrocytes that contains 2 to 3 mM, extra cellular fluid of glutathione<sup>11-18</sup>. In the lens it is highly concentrated at the periphery but less in the interior and so on<sup>19</sup>. Thus organ of vision contains the richest concentration of glutathione, particularly in the cortex of lens, anterior epithelium of the cornea, optic fasciculus and retina. In vertebrates from mammals to fish, the concentration of GSH in eyes, especially in lens is commonly very

high reaching several times of that in blood & twice that in the liver<sup>20</sup>.

Lower glutathione levels are implicated in many diseases associated with aging, these diseases include Alzheimer's disease, Parkinson's, atherosclerosis<sup>21</sup>, neuro-degenerative diseases. Lower levels also correspond to poor survival of AIDS<sup>22</sup> patients. Glutathione protects against the complications of diabetes<sup>23</sup>, and lung disease. But is impaired in alcoholic hepatitis as well as in viral hepatitis A, B, and C. Raised glutathione levels can restore liver function<sup>24</sup>. Tranexamic acid (Trans-4 $\alpha$  aminomethylcyclohexane Carboxylic acid<sup>25</sup>) is the derivative of an amino acid lysine. This drug inhibits the proteolytic activity of plasmin in the cleavage of fibrinogen and a series of other proteins involved in coagulation. This drug also inhibits the conversion of plasminogen to plasmin by plasminogen activators<sup>26</sup>.

Tranexamic acid is indicated in; Bleeding tendencies in which systemic hyperfibrinolysis is considered to be involved. Such as (abnormal bleeding during or after operation, Leukemia, aplastic anemia, purpura, etc).

- Abnormal bleeding in which local hyperfibrinolysis is considered to be involved. These includes Pulmonary hemorrhage, nasal hemorrhage, vaginal hemorrhage, renal hemorrhage, abnormal bleeding during or after prostate surgery.
- Symptoms such as erythema, swelling or pruritus in diseases, eczema condition, urticaria, and drug eruption or toxicoderma.
- Symptoms such as pharynganglia, redness, hyperemia or swelling tonsillitis, pharyngolaryngitis.
- Pain in the oral cavity and mucosal aphtha in stomatitis.

## MATERIALS AND METHODS

Acetyl Tranexamic acid and Phthaloyl Tranexamic acid were prepared according to a published method<sup>27</sup>. All the HPLC grade chemicals (i.e Sodium Hydroxide, Phthalic anhydride, L-Glutathione, Disodium Hydrogen Phosphate Dihydrate (Na<sub>2</sub>HPO<sub>4</sub> · 2H<sub>2</sub>O) and HCl 35%) were purchased and used as without further purification.

### Preparation of Glutathione (GSH) and Stock Solution

20 ml of 1 mM GSH (M.W.307.4) solution was prepared by dissolving 6.2g GSH in 0.1N hydrochloric acid. 10<sup>-2</sup> M DTNB (M.W 396.4) solution was prepared by dissolving 79.28 mg in 20ml. 0.1 M phosphate buffer (pH 7.6) 1000 ml of was prepared by dissolving 17.799 g in 1000 ml of doubly distilled water. pH was adjusted to 7.6 with 0.1 N HCl.

### Preparation of GSH Standard Curve

100, 200, 300, 400 and 500  $\mu$ L of 1 mM GSH solution were diluted with phosphate buffer (pH 7.62), The final concentrations of the diluted GSH solutions were 0.1, 0.2, 0.3, 0.4 and 0.5 mM. A standard curve for GSH was obtained by slight modification of a standard method of ELLMAN<sup>28</sup> which was carried out as follows.

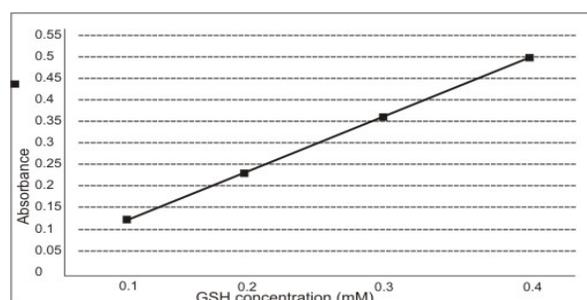
200  $\mu$ l of each GSH diluted solutions obtained were added to 2.3 ml of Phosphate Buffer (pH 7.62), followed by the addition of 500  $\mu$ l of 10<sup>-2</sup> M DTNB.

A blank was prepared by mixing 2.5 ml of Phosphate Buffer (PH 7.62) with 500 $\mu$ l of DTNB solution. All mixtures were shaken thoroughly and incubated for five minutes. After which the absorbance at 412 nm were measured. The real absorbance for each GSH concentration was obtained by subtracting the absorbance for DTNB from the absorbances for GSH plus DTNB

mixtures. A standard curve obtained for GSH as shown in FIG.1

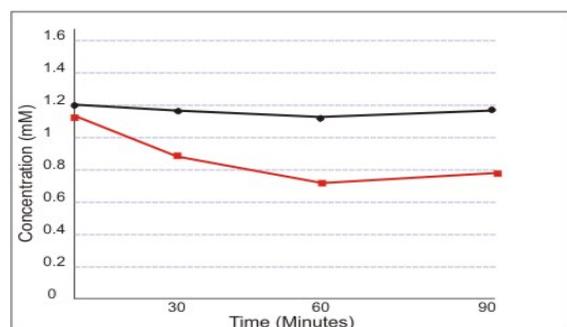
### Effect of Tranexamic Acid on the Chemical Status of Glutathione

To 1000  $\mu$ l of 1 mM GSH in a test tube, 1 ml of 1 mM Tranexamic acid solution was added. The final concentration of the GSH and Tranexamic acid in a test tube was 500  $\mu$ M. A control for 1 mM GSH with the final concentration of 500  $\mu$ M was also prepared.



**FIG. 1: Standard Curve for GSH.**

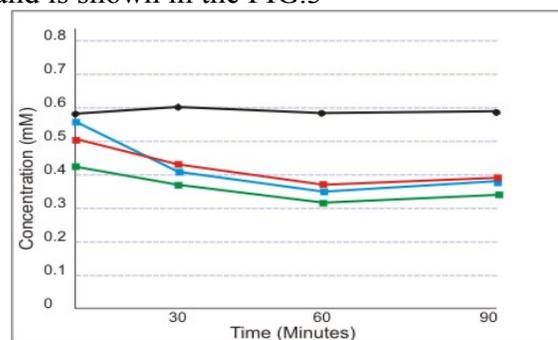
The effect of Tranexamic acid on the chemical status of GSH was studied in terms of determination of concentration of GSH in the mixture containing 500  $\mu$ M Tranexamic acid by a well-known ELLMAN's<sup>28</sup> method, as mentioned above (standard curve for GSH). The absorbencies were read at 0, 30, 60 and 90 minutes after mixing. The concentration of GSH was determined from the GSH standard curve and is shown in the FIG.2



**FIG. 2: Effect of Tranexamic Acid on the concentration of GSH**

### Effect of different Concentrations of Tranexamic acid on the chemical status of Glutathione

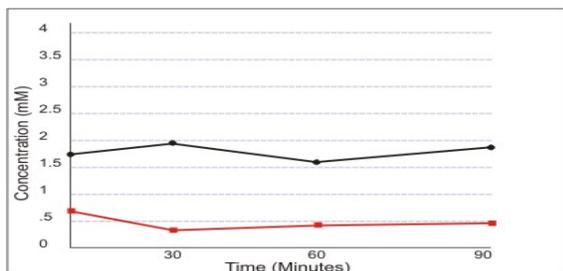
To 1000  $\mu$ l of 1 mM GSH in three separate test tubes, 1000  $\mu$ l, 800  $\mu$ l and 600  $\mu$ l of 1 mM, Tranexamic acid solutions were added and further diluted with phosphate buffer (pH 7.6) to 1 ml. The tubes were shaken and incubated for 5 minutes with the final concentrations of GSH in each test tube were 500  $\mu$ M and that of Tranexamic acid were 500  $\mu$ M and 400  $\mu$ M and 300  $\mu$ M respectively. A control for 1 mM GSH with the final concentration of 500  $\mu$ M was also prepared. The effect of different concentration of Tranexamic acid on the chemical status of GSH was studied for the determination of concentration in the mixture by a well-known ELLMAN's<sup>28</sup> method as mentioned above (standard curve for GSH). The absorbances were read at 0, 30, 60, and 90 minutes after mixing. GSH concentration was determined from the GSH standard curve and is shown in the FIG.3



**FIG. 3: Effect of Tranexamic acid on the chemical status of the GSH.**

### Effect of Acetyl Tranexamic Acid<sup>17</sup> on The Chemical Status of Glutathione

To 1000  $\mu$ l of 1 mM GSH in a test tube, 1 ml of 1mM Acetyl-tranexamic acid solution was added. The final concentration of the GSH and Acetyl-tranexamic acid in a test tube was 500  $\mu$ M. A control for 1 mM GSH with the final concentration of 500  $\mu$ M was also prepared.

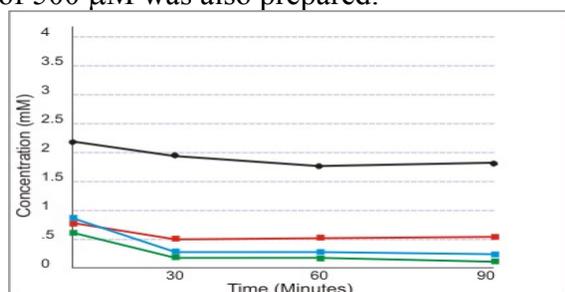


**FIG. 4: Effect of Acetyltranexamic acid on the concentration of GSH.**

The effect of Acetyl Tranexamic acid on the chemical status of GSH was studied in terms of determination of concentration of GSH in the mixture containing 500  $\mu\text{M}$  Acetyl tranexamic acid by a well known ELLMAN's<sup>28</sup> method, as mentioned above (standard curve for GSH). The absorbencies were read at 0, 30, 60 and 90 minutes after mixing. The concentration of GSH was determined from the GSH standard curve and is shown in the FIG.4

#### Effect of different concentrations of acetyl tranexamic acid on the chemical status of Glutathione

To 1000  $\mu\text{l}$  of 1 mM GSH in three separate test tubes, 1000  $\mu\text{l}$ , 800  $\mu\text{l}$  and 600  $\mu\text{l}$  of 1 mM. Acetyl-tranexamic acid solutions were added and further diluted with phosphate buffer (pH 7.6) to 1 ml. The tubes were shaken and incubated for 5 minutes with the final concentrations of GSH in each test tube were 500  $\mu\text{M}$  and that of Acetyl-tranexamic acid were 500  $\mu\text{M}$  and 400  $\mu\text{M}$  and 300  $\mu\text{M}$  respectively. A control for 1 mM GSH with the final concentration of 500  $\mu\text{M}$  was also prepared.

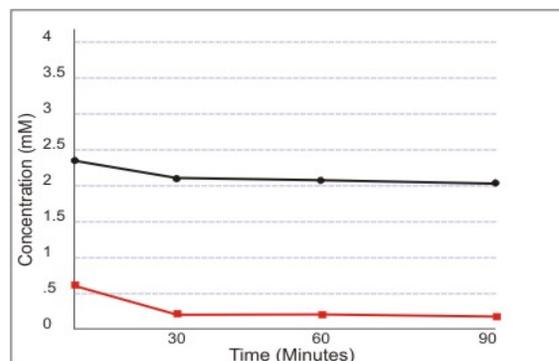


**FIG. 5: Effect of different concentrations of Acetyl tranexamic acid on the chemical status of GSH.**

The effect of different concentration of Acetyl-tranexamic acid on the chemical status of GSH was studied for the determination of concentration in the mixture by a well-known ELLMAN's<sup>28</sup> method as mentioned above (standard curve for GSH). The absorbances were read at 0, 30, 60, and 90 minutes after mixing. GSH concentration was determined from the GSH standard curve and is shown in the FIG.5

#### Effect of Phthaloyl Tranexamic Acid on the Chemical Status of Glutathione<sup>17</sup>

To 1000  $\mu\text{l}$  of 1 mM GSH in a test tube, 1 ml of 1 mM Phthaloyl-tranexamic acid solution was added. The final concentration of the GSH and Phthaloyl-tranexamic acid in a test tube was 500  $\mu\text{M}$ . a control for 1 mM GSH with the final concentration of 500  $\mu\text{M}$  was also prepared.



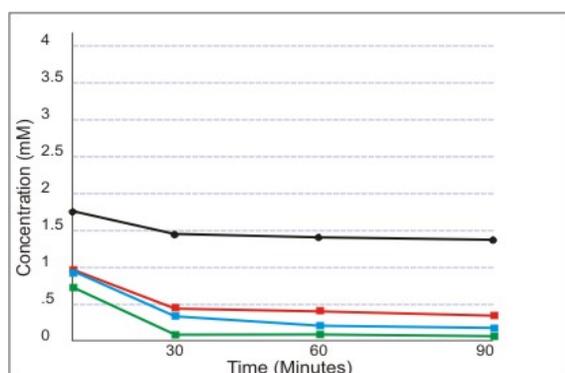
**FIG. 6: Effect of Phthaloyltranexamic acid on the concentration of GSH**

The effect of Phthaloyl-tranexamic acid on the chemical status of GSH was studied in terms of determination of concentration of GSH in the mixture containing 500  $\mu\text{M}$  Phthaloyl-tranexamic acid by a well-known ELLMAN's<sup>28</sup> method, as mentioned above (standard curve for GSH). The absorbencies were read at 0, 30, 60 and 90 minutes after

mixing. The concentration of GSH was determined from the GSH standard curve and is shown in the FIG.6

#### **Effect of different concentrations of Phthaloyl Tranexamic Acid on the chemical status of Glutathione**

To 1000  $\mu$ l of 1 mM GSH in three separate test tubes, 1000  $\mu$ l, 800  $\mu$ l and 600  $\mu$ l of 1 mM. Phthaloyl-tranexamic acid solutions were added and further diluted with phosphate buffer (pH 7.6) to 1ml. The tubes were shaken and incubated for 5 minutes with the final concentrations of GSH in each test tube were 500  $\mu$ M and that of Phthaloyl Tranexamic acid were 500  $\mu$ M and 400  $\mu$ M and 300  $\mu$ M respectively. A control for 1-mM GSH with the final concentration of 500  $\mu$ M was also prepared.



**FIG. 7. Effect of different concentrations of phthaloyltranexamic acid on the chemical status of the GSH.**

The effect of different concentration of Phthaloyl-tranexamic acid on the chemical status of GSH was studied for the determination of concentration in the mixture by a well-known ELLMAN'S<sup>28</sup> method as mentioned above (standard curve for GSH). The absorbances were read at 0, 30, 60, and 90 minutes after mixing. GSH concentration was determined from the GSH standard curve and is shown in the FIG.7.

## **RESULTS AND DISCUSSION**

Effect of Tranexamic acid, and its derivative i.e. Acetyltranexamic acid & Phthaloyl tranexamic acid, on the glutathione were studied in term of the determination of concentration of Glutathione.

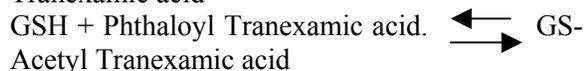
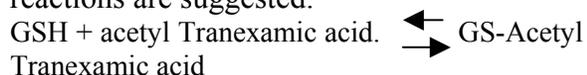
Tranexamic acid caused an increase in the concentration of GSH. This increase and decrease in the concentration of GSH was time dependent are shown in FIG. 2 and 3. Effect of Acetyltranexamic acid was studied, this complex caused decrease in the concentration of GSH, while concentration of GSH decreases slightly during 30, 60, & 90 minutes as shown in the FIG..4 and 5. Effect of Phthaloyl tranexamic acid was studied, this complex caused decrease in the concentration of GSH, while concentration of GSH decreases slightly during 30, 60, and 90 minutes as shown in the FIG.6 and 7.

There is increasing interest in Glutathione due to its varied Physiological and pharmacological properties including detoxification through participation in the redox system, activation of SH – enzymes, co-enzymatic action and conjugation, for instance formation of mercapturic acid.<sup>29</sup>, as we know that GSH has strong anti-oxidant property, while Tranexamic acid is the derivative of amino acid lysine. This drug shows the inhibition of the proteolytic activity of plasmin and the conversion of plasminogen to plasmin by plasminogen activators<sup>30-34</sup>. They show good hemostatic action, anti- allergic<sup>40</sup> and anti-inflammatory effect.<sup>35-38</sup> & anti cancer activity<sup>41-42</sup>, while glutathione is also known as NATURAL DRUG<sup>1</sup> and best ANTI-OXIDANT<sup>39</sup>, thus it was of interest to study the interaction of these drugs in vitro to establish further scientific data. This scientific data about the interaction and the effect of Tranexamic acid, on the chemical

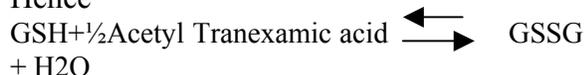
modulation of GSH will enable us to understand further the role of Tranexamic acid and GSH and strengthen our knowledge about their therapeutic uses in many diseases.

### Effect of Tranexamic Acid, Acetyl Tranexamic Acid and Phthaloyl Tranexamic Acid on the chemical status of GSH

In this research work the effect of Tranexamic acid, acetyltranexamic acid on the chemical status of GSH was also studied in terms of determination of concentration of GSH at  $\lambda_{\max}$  412 nm. This  $\lambda$  412nm is being used for the determination of GSH concentration especially thiols in samples by a well-known ELLMAN'S<sup>28</sup> method. During this study, it is found that Acetyltranexamic acid and Phthaloyltranexamic acid was more effective in lowering of thiols than Tranexamic acid itself. Acetyltranexamic acid and Phthaloyltranexamic acid probably played a role to oxidize GSH to GSSG. This perhaps has occurred in the presence of air oxygen. The following sequences of reactions are suggested.



Hence



To summarize Acetyltranexamic acid and Phthaloyltranexamic acid oxidized GSH to GSSG in the presence of air oxygen. Therefore it is essential that thiols especially GSH concentration determination be done in the atmosphere to avoid oxidation of thiols

or GSH to a greater extent. This will give us correct results instead of false results. These results also suggested that there was a possibility of formation of intermediate or conjugate between Acetyltranexamic acid and Phthaloyltranexamic acid and GSH. However it was not possible to estimate or determine those conjugates under those conditions. Since both GSH and Phthaloyl Tranexamic acid are biologically active compounds. It was of interest to study the possible interaction of these two compounds in vitro as a model of in vivo interaction.

GSH determination may be an important indicator of pharmacological action or toxic symptoms of chemical substances or a drug. Further study is required to understand and confirm reactions given above for further role GSH and therapeutic evaluation of Acetyl tranexamic acid and Phthaloyltranexamic acid in many diseases. This will help to explain and understand a variety of pharmacological properties of this complex.

### ACKNOWLEDGEMENT

I am grateful to Zafa Pharmaceuticals, Pvt Ltd. Pakistan for providing Tranexamic acid for this study.

### REFERENCES

1. Lands LC, Grey VL, Smountas AA. Effect of Supplementation with a cysteine donor on muscular performance. *J Appl Physiol* 1999; 87 : 1381- 5
2. Alton Meister. Glutathione, Ascorbate, and Cellular Protection, cancer research 1969s, 1975s, 1994(5),
3. Meister A, & Tate S. S. Glutathione and related gamma- glutamyl compounds; *Biosynthesis & utilization*, *Annu. Rev. Biochem* 1976; 45: 559-604.
4. Sies H & Wendel A. Glutathione functions in liver & kidney. Springer Verlag publishers Berlin. 1978; Page 43
5. Kisower N S & Kisower E M, The Glutathione status of cells, *Int. Rev. cytol.* 1978; 54-190.
6. Brian K. *Mutation Research* 1988; 202- 343.

7. Sies H & Wendel A. Functions of Glutathione in Liver and Kidney, Springer Verlag Publisher, 1978; Berlin, Heidelberg: New York.
8. Chasseaud L F. et al. The role of Glutathione and S – Transferase in the metabolism of chemical carcinogens and other electrophilic agents. *Advances in Cancer Research*, 1979; 29: 175 – 274.
9. Sies H, Wah Llander A, Waydhas C, H Soboll S, and Haberle D *Adv. Enzyme Reg.* 1980; 18, 303.
10. Hardin E, Betal M J, *Med. Sci.* 1954; 73: 228.
11. Fisher M M. and Kerly M, *J.Physiol*, 1964; 174 ,273
12. Refsvik T, and Norseth, T. *Acta Pharmacol. Toxicol*, 1975; 36: 67.
13. Refsvik T, *Acta. Pharmacol. Toxicol*, 1978; 42:135.
14. Sies H, Koch O R, Martin E, Boveris A & Febs Lett.1979; 103- 287.
15. Eberle D, Clarke R, and Kaplowitz N, *J. Biochem* 1981; 265: 2115.
16. Akerboom T P M, Bilzer M, and Sies H, *J. Biochem* 1982 b; 257 : 4248.
17. Ballatori N, and Clarkson T W, *Science*, (Washington D. C) 1982; 216: 61.
18. Ballatori N, and Clarkson T W, *J. Physiol* 1983; 244: G 435.
19. Ashgar K, Reddy B G, and Krishna G, *J. Histechem Gytochem* 1975; 23:774.
20. Oguchi M et al.: *J. of Japanese Society of Ophthalmology* 1962; 66, 1488.
21. *Journal of Clinical Epidemiology* 47:21-26 1994.
22. *Proceedings of National Academy of Science, USA* 94:1967-72, 1997.
23. *Clinical Science* 91:575-582, 1996.
24. *American Journal of Gastroenterology* 91:2569-2573, 1996.
25. Akinori Nakanishi (D.G.) *Japan Pharmaceutical refrence*, 1999; 5: 139-144,
26. Levy J H, *Anti-fibrinolytics = Aminocaproic acid, Tranexamic acid, and Apotinin. The Intern Journal of Anesthesiology*, 1997; 2.
27. M.Fayyaz K, M. Farid Khan et al; *Comparative evaluation of some new tranexamic acid derivatives and their copper (II) complexes for anti-tumor, analgesic, bactericidal and fungicidal activities. Pak J of Pharmaceutical Sciences*, 2002; 15: 55-62.
28. Ellman G L, *Arch, Biophys* 1959; 82, 70.
29. Hayashi O, In 'progress in study on glutathione', published by Shindan to Chiryosha. P.I. 1969
30. Abiko, *Med Pharm.* 1976; 10:1- 7.
31. Iwamoto M, *Thrombos Diathes.Hemorrh* 1975; 33:573.
32. Makus G, et al., *Biochem* 1979; 254:1211.
33. Abiko Y, et al., *Biochem. Biophys. Acta.* 1969; 185:424.
34. Abiko Y, et al., *Biochem. Biophys. Acta.* 1970; 214:411.
35. Yamada et al., *Report of plasmin study group* 1974; 14: 364.
36. Kimura et al., *Allergy* 1966; 15:9, 755.
37. Kondo, *Report of plasmin study Group* 1966; 6, 36.
38. Yamazaki et al., *Dolia pharmacol. Japanica*, 1967; 63:6,560.
39. *Biochemical pharmacology:* 472113-2123 (1994).
40. Munka et al., *Nishnihon. J.Dermatol.* 331:2, 141,(1969)
41. Leuthauser, S.W.C, Oberay, T.D., Soreanson, J.R.J and Ramakrishna, K. (1981); *JNAT, cancer Inst*; 66, 1077.
42. Sorenson J.R.J. and Oberley, L.W. (1983): *Biol. Trace elements Res.* 5, 257